UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: March 28, 2013

SUBJECT: **Dinotefuran:** Review of Developmental Neurotoxicity Study.

PC Code: 044312
Decision No.: 442170
Petition No.: NA
Risk Assessment Type: Single Chemical
TXR No.: 0055570
MRID No.: See table below

DP Barcode: 384323
Registration No.: 86203-8
Regulatory Action: Section 3 Registration
Case No.: NA
CAS No.: 165252-70-0
40 CFR: §180.603

FROM: Sheila Healy, Toxicologist
Risk Assessment Branch III
Health Effects Division (7509P)

THRU: Christine Olinger, Chief
Risk Assessment Branch III
Health Effects Division (7509P)

TO: Venus Eagle and Rita Kumar/RM 01
Insecticide/Rodenticide Branch
Registration Division (7505P)

I. CONCLUSIONS

The toxicology study submitted to meet the conditional requirement for registration of dinotefuran has been reviewed for completeness and general acceptability. This study has been included in the hazard characterization and hazard identification for risk assessment.

II. ACTION REQUESTED

Review the submitted study for the requested registration action and risk assessment.

III. MRID Summary Table

Guideline #; Study Type	MRID	Comments
870.6300; Oral developmental neurotoxicity study (rat)	48291601	New DER

DATA EVALUATION RECORD
MTI-446 DINOTEFURAN
PC Code: 044312
TXR#: 0055570
MRID#: 48291601

Oral (Diet) Developmental Neurotoxicity Study of
MTI-446 (Dinotefuran) in Crl:CD(SD) Rats
OPPTS 870.6300

Prepared for

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Principal Reviewer Daniel Ewald Date 1/31/11

Secondary Reviewer William Burnam, M.S. Date 2/1/11

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Contract Number: EP-W-10013
Work Assignment No.: WA-0-01

Task No.: 0-1-26
EPA Reviewer/WAM: Healy/Ottley

This review may be altered by EPA subsequent to the contractors' signatures above.

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EPA Reviewer: Sheila Healy, Ph.D **Signature:**
Risk Assessment Branch 3, Health Effects Division (7509P) **Date:** 4/3/2013
EPA Secondary Reviewer: Whang Phang, Ph.D **Signature:**
Risk Assessment Branch 3, Health Effects Division (7509P) **Date:** 4/3/13

DATA EVALUATION RECORD

STUDY TYPE: Oral Developmental Neurotoxicity Study

PC CODE: 044312 **DP BARCODE:** 384323
TXR#: 0055570

TEST MATERIAL (PURITY): Dinotefuran (99.5% purity)

SYNONYMS: MRI-446

CITATION: Hoberman, A (2010) Oral (Diet) Developmental Neurotoxicity Study of MTI-446 (Dinotefuran) in Crl:CD(SD) Rats. Charles River Laboratories, 905 Sheehy Drive, Horsham, PA. Sponsor Study Number SRY00002, October 19, 2010 MRID 48291601

SPONSOR: Mitsui Chemicals Agro, Inc. Tokyo, Japan

EXECUTIVE SUMMARY: The purpose of this study was to provide information for use in evaluating the potential for functional or histopathological neurotoxic effects in offspring after exposure to MTI- 446 (Dinotefuran) *in utero* and/or via maternal milk during the lactation period.

One hundred presumed pregnant Crl:CD(SD) rats were randomly assigned to four exposure groups, 25 rats per group. The test substance in the diet, MTI-446 (Dinotefuran), or vehicle were offered continuously on days 6 of gestation (GD 6) through day 21 of lactation (PND 21) until sacrifice at exposures of 0 , 1000, 3000 or 10000 ppm. Dietary exposures were equivalent to 0, 79.4, 237.4 or 784.1 mg/kg/day during gestation and 0, 158.0, 500.7 or 1642.9 mg/kg/day during lactation. All maternal rats were sacrificed after completion of the 21-day postpartum period and a gross necropsy was performed. The following parameters were evaluated: viability, clinical observations, detailed clinical observations, maternal body weight and body weight changes, maternal behavior, feed consumption values, necropsy observations, the number and distribution of corpora lutea, implantation sites, uterine contents, litter size and pup viability.

On day 4 (PND 4) all litters were reduced to ten pups each by random selection; 25 appropriately sized litters per group were selected for examination on study. Ten pups of each sex and dosage group were randomly selected for brain weight/dimensions determination and neurohistopathology (Subsets 1 and 4). Litters with fewer than nine pups were not assigned to study, but retained until not needed. One male and one female pup/litter (up to 25/sex/subset) were assigned to each evaluation as follows:

Subset 1 - PND 21 brain weight/gross dimensions (all dose groups) and neurohistopathology and microscopic brain measurements (0 and 10000 ppm dose groups only)

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Subset 2 - Watermaze and passive avoidance
Subset 3 - Motor activity and acoustic startle habituation
Subset 4 - Brain weight/gross dimensions (all dose groups) and neurohistopathology and microscopic brain measurements (0 and 10000 ppm dose groups)
Subset 5 - One pup per sex used for replacement of rats found dead or sacrificed prior to scheduled termination during PND 4 through 21.

Rats were observed for viability at least twice daily during the postweaning period and clinical observations and general appearance once weekly during the postweaning period. Additionally, rats assigned to Subsets 2 and 3 were examined for gross signs of toxicity and clinical observations; and rats assigned to Subset 4 were examined by an individual unaware of each rat's dosage group for detailed clinical observations. Body weights for male and female rats were recorded weekly during the postweaning period and prior to sacrifice. Pup body weights were recorded on PNDs 0, 4, 7, 11, 13, 17 and 21. Food consumption values for male and female rats were recorded weekly during the postweaning period and on the day of preputial separation or vaginal patency for subsets 2 through 4.

Subset I pups selected for brain weight/dimension determination and neurohistopathological evaluations were sacrificed and processed on PND 21. All pups in Subset 1 were examined for gross lesions. Subsets 2 and 3 rats were sacrificed after completion of the postweaning behavioral evaluations. Subset 4 rats randomly selected for neurohistopathological evaluations were sacrificed on PND 69 and those not selected for these procedures were sacrificed and examined for gross lesions. Subset 5 pups were sacrificed on PND 21 and examined for gross lesions.

All F0 generation female rats survived to scheduled sacrifice. No adverse clinical observations related to exposure to MTl-446 occurred. Body weight gains for intervals GD 6 to 9and GD 6 to 20 were significantly reduced, 45% and 11%, respectively, in the 10000 ppm exposure group compared to the control group value; however, this body weight decrement was not sustained and body weights on GD 20 in all treatment groups were essentially equivalent to control values. Body weight gains during the lactation period did not differ significantly among the treatment groups. While maternal body weights were significantly reduced in the 10000 ppm exposure group on days 11 and 16 of lactation (LD 11 and 16), this ~4% decrement is not considered toxicologically significant. Exposure to MTI-446 at up to 3000 ppm during gestation and lactation did not affect body weight gains or body weights. Absolute and relative food consumption values were generally comparable among the exposure groups.

No maternal gross lesions related to exposure to MTI-446 occurred.

No clinical and no necropsy observations in the Fl generation pups were attributed to maternal exposure to the test substance at doses as high as 10000 ppm.

No mortality related to maternal exposure to MTI-446 occurred in the male or female rats postweaning. All maternal rats survived to scheduled sacrifice. All male offspring rats survived to scheduled sacrifice. One female rat in the 10000 ppm maternal exposure group was found dead on postnatal day PND23, another was found dead on PND 26 and two female rats in the 0 ppm exposure group were missing on PND 21 or 22 and another was sacrificed due to adverse

clinical observations on PND 58. These single events were not considered related to MTI-446. All other rats survived to scheduled sacrifice.

All clinical and necropsy observations for the F1 generation male and female rats were considered to be unrelated to the test substance. Average body weights, body weight gains and absolute and relative feed consumption values in the male and female rats were unaffected by maternal exposure to MTI-446 at exposures up to 10000 ppm.

Maternal exposure to MTI-446 at concentrations as high as 10000 ppm did not affect the day of vaginal patency or preputial separation. Motor activity was not affected by maternal exposures to MTI-446 as high as 10000 ppm. Maternal exposure to MTI-446 as high as 10000 ppm did not affect learning and memory as evaluated in a passive avoidance paradigm.

There were no microscopic changes in the tissues evaluated histologically that were considered to have been the result of test substance administration. The changes that were observed were typical of those that occur spontaneously or as incidental findings in rats of this age and strain.

No statistically significant inter-group differences were noted for brain weights or for gross brain dimensions on days 21 and 69 postpartum. No treatment-related microscopic lesions were present in any of the tissues examined in the central and peripheral nervous systems at 10000 ppm.

> The no-observed-adverse-effect level (NOAEL) for both functional and histopathological developmental neurotoxicity in the F1 generation rats whose mothers were exposed to MTI-446 was 10000 ppm, the highest dose tested. This dietary concentration provided an average maternal dose level of 784 mg/kg/day during the gestation and 1643 mg/kg/day during the lactation period. Decreases in body weight gain are not considered adverse since they are not sustained and there is no difference in body weight among dose groups during gestation , lactation or postweaning. Therefore, a NOAEL for all toxicological effects is also established as 10000 ppm, the highest dose tested.

This study is classified **acceptable/guideline** and satisfies the guideline requirement for a developmental neurotoxicity in rats

COMPLIANCE: A signed and dated GLP statement was provided. There was a signed and dated statement of no claims of data confidentiality. The study was performed in compliance with the requirements of OPPTS 870.6300 and OECD 426 test guidelines.

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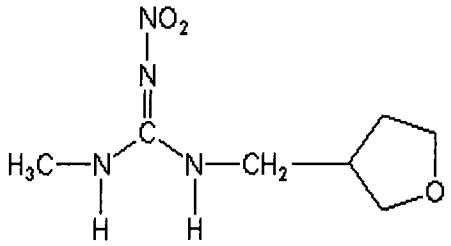
I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Dinotefuran – MTI-446

Description:	White powder
Lot/batch #:	2200210
Purity:	99.5
Compound stability:	For at least 14 and 21 days, stability was determined for the lowest concentration after storage at ambient temperature.
CAS # of TGAI:	165252-70-0
Structure:	

2. Vehicle: The vehicle for the test substance was the diet (further details below).

3. Test animals:

Species:	Rat – male and female	
Strain:	Crl:CD(SD)	
Age/weight at study initiation:	188 – 230 g	
Source:	Charles River Laboratories, Inc., Portage, Michigan, USA	
Housing:	Individually except during mating and lactation periods..	
Diet:	Certified Rodent Diet®#5002, *ad libitum.*	
Water:	Local water, processed by passage through a reverse osmosis membrane, was available to the rats *ad libitum.* Chlorine was added to the processed water as a bacteriostat.	
Environmental conditions:	**Temperature:**	18-23°C
	Humidity:	30% - 67%
	Air changes:	10/hour
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Five days	

B. STUDY DESIGN:

1. In life dates:
Start: August 18, 2009 (Arrival of F0 Generation of Rats)
End: December 1, 2009 (Last scheduled sacrifice)

2. Animal assignment: The maternal animals were mated and assigned to study> the test substance was administered to the maternal animals from gestation day 6 through postnatal day 21. Maternal animals were sacrificed on PND 21. F1 pups remained on study until PND 69 (study termination).

3. Mating procedure: Females were paired 1:1 with males of the same strain and source fir a maximum of 7 days. Each female was examined daily during the mating period to identify sperm cells in a vaginal smear or the presence of a copulatory plug. The day that sperm or a plug was found was designated gestation day 0 (GD 0). Pregnant dams were housed in nesting boxes no later than GD 20 where they were maintained through lactation.

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4. **Animal assignment**: Animals were assigned in a randomized pattern (pages 32-35 of the study). Some of the animals from the F1 generation were also assigned to measure neurohistopathology and brain weights/dimensions. Fl generation pups selected for Subset 5 were not individually identified. Pups were sacrificed on PND 21.
   Subset groups were categorized by the following:
   Subset 1 - PND 21 brain weight/gross dimensions (all dose groups) and neurohistopathology and microscopic brain measurements (0 and 10000 ppm dose groups only)
   Subset 2 - Watermaze and passive avoidance
   Subset 3 - Motor activity and acoustic startle habituation
   Subset 4 - Brain weight/gross dimensions (all dose groups) and neurohistopathology and microscopic brain measurements (0 and 10000 ppm dose groups)
   Subset 5 - One pup per sex used for replacement of rats found dead or sacrificed prior to scheduled termination during PND 4 through 21.

Table 1 - Study Design

Experimental Parameter	Subset	Dose (ppm)			
		0	1000	3000	10000
Maternal Animals (F0)					
No. of dams assigned	NA	25	25	25	25
No. of dams pregnant	NA	23	25	23	24
Mean daily intake gestation (mg/kg/day)	NA	0	79.4	237.4	784.1
Mean daily intake lactation (mg/kg/day)	NA	0	158.0	500.7	1642.9
Offspring (F1)					
PND 21 Brain weight /dimensions (all dose groups) and neurohistopathology (0 and 10000 dose groups only)	1	10/sex 10/sex	10/sex NA	10/sex NA	10/sex 10/sex
Watermaze and passive avoidance	2	23/sex	25/sex	23/sex	24/sex
Motor activity and acoustic startle habituation	3	23/sex	25/sex	23/sex	24/sex
Brain weight /dimensions (all dose groups) and neurohistopathology (0 and 10000 dose groups only)	4	10/sex 10/sex	10/sex NA	10/sex NA	10/sex 10/sex

Source: pp. 18-20, of the Study Report

Rats were permanently identified using Monel® self-piercing ear tags. Male rats were given unique permanent identification numbers upon assignment to the Test Facility's breeder male rat population. Female rats were assigned temporary numbers at receipt and given unique permanent identification numbers when assigned to the study on the basis of DG 0 body weights. Cage tags were marked with the study number, permanent rat number, sex, test substance identification, generation, group number and dosage level.

On PND 4, pups retained for study were individually identified within the litter by tail tattoo indicating their respective sequence numbers on that day. Ink was injected under the skin of the tail to mark the pups. Pups selected for motor activity (Subset 3) were permanently identified by tail tattoo by PND 13. Whenever possible, all data were evaluated in terms of the litter before weaning. On PND 21, each rat selected for continued observation was identified by tail tattoo.

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F1 generation pups may have been exposed to the test substance *in utero* during gestation and were exposed via maternal milk and maternal feed during the postpartum period. Fl generation rats were not directly exposed to the test substance following weaning.

5. **Dose selection rationale:** A prior dose range-finding study conducted at dose levels of 1000, 3000 and 10000 ppm led to no adverse findings in maternal F0 generation rats at 10000 ppm (equivalent to an average dose level of 1035 mg/kg/day). However, the NOAEL for general toxicity following exposure of the F1 generation was 3000 ppm. Direct exposure through the diet and milk resulted in a reduction in body weight on days 13 through 57 (males) or 64 (females) in F1 generation rats in the 10000 ppm exposure group. Therefore, 10000 ppm was considered a suitable high dose level for this guideline study.

6. **Dosage administration:** All doses were administered in the diet beginning on GD 6 through PND 21. Pups were not dosed directly.

7. **Diet preparation and analysis** Formulation (diets) were prepared once bi-weekly at the Testing Facility and shipped immediately for analysis (Appendix 5 of study report). Stability of dinotefuran formulations at a concentration of 1000 ppm was evaluated for storage stability at 22 ± 5°C for 15 and 21 days..

Results:
Homogeneity analysis: Neither details nor data were not provided for homogeneity sampling.

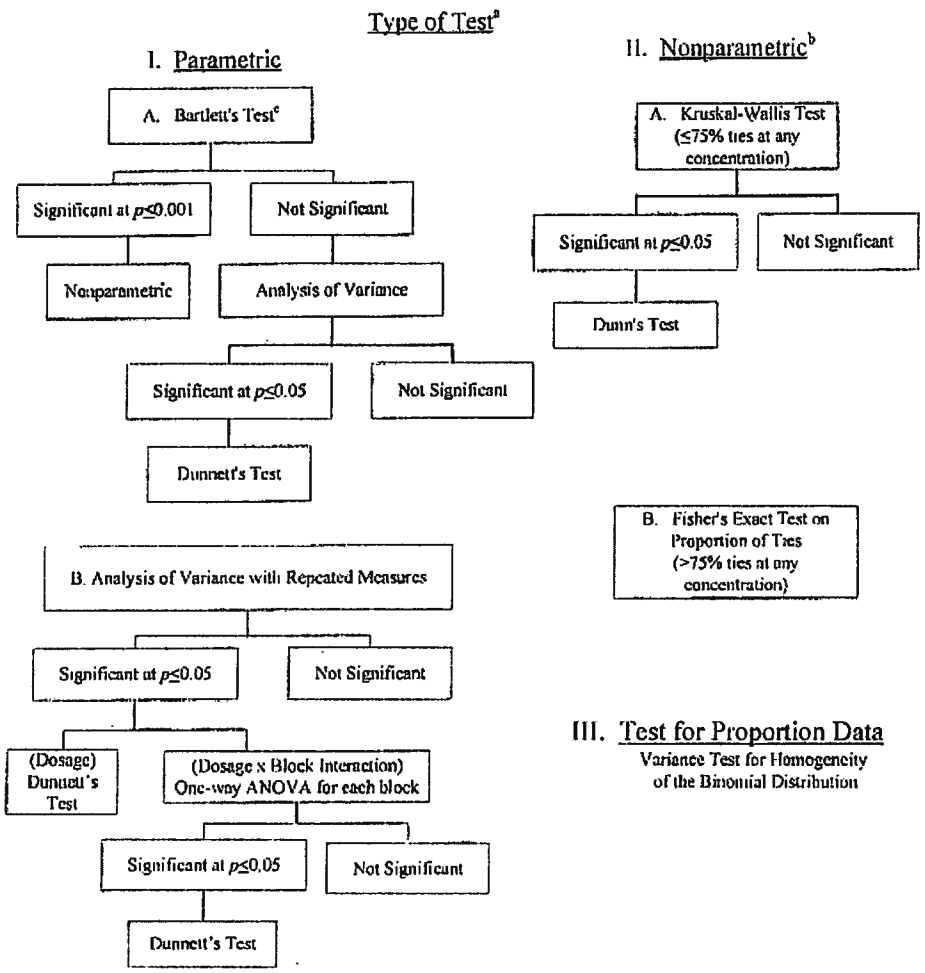
Stability analysis: (Range as mean % of nominal): 98-99.4%

Concentration analysis: (Range as mean % of nominal): 94.7-100.6%

The analytical data indicated that the variation between nominal and actual dosage to the animals was acceptable.

6. **F1 Generation:**

7. **Statistical Analysis:**
Averages and percentages were calculated. Litter values were used where appropriate. The following schematic represents the statistical analyses of the data:



a. Statistically significant probabilities are reported as either $p \leq 0.05$ or $p \leq 0.01$.
b. Proportion data are not included in this category.
c. Test for homogeneity of variance.

Source: p. 45 of the Study Report.

The Reviewer considers these analyses used to be appropriate.

C. OBSERVATIONS:

1. In-life observations:

a. **Maternal animals:** Rats were observed for viability at least twice each day of the study and for clinical observations and general appearance weekly at approximately the same time each week during the pre-exposure period and on GD 0. The rats were also examined for detailed clinical observations daily beginning on GD 6. Observations were made approximately the same time each day. The detailed clinical observations were conducted by an observer unaware of the dosage group assignment of the rats. These observations included (but were not limited to): general appearance (skin, fur, changes in eyes, eyeballs and mucous membranes), body position and posture (e.g., hunchback posture), autonomic nervous system function (lacrimation, piloerection, pupil diameter, respiration, excretion), motor coordination, ambulatory abnormalities, reaction to being

handled and to environmental stimulation, nervous system (tremor, convulsion, muscular contractions), changes in exploratory behavior, ordinary behavior (changes in grooming, head shaking, gyration), abnormal behavior (autophagia, backward motion, abnormal vocalization) and aggression.

Body weights were recorded weekly during the pre-exposure period, on GD 0 and daily all other periods, including the day of sacrifice. Food consumption values were recorded on GD 0 and daily during all other periods. Because pups begin to consume maternal feed on or about PND 13, feed consumption values were not tabulated after PND 13.

Rats were evaluated for adverse clinical signs observed during parturition, duration of gestation (DG 0 to the day the first pup was observed), litter sizes (all pups delivered), live litter *size* (live born pups only) and pup viability at birth. Maternal behavior was evaluated on PND 0 (Birth), 4, 7, 13 and 21.

b. **Offspring:**

1. **Litter observations**: Day 0 of lactation was defined as the day of birth and was also the first day on which all pups in a litter were individually weighed (pup body weights were recorded after all pups in a litter were delivered and groomed by the dam). Each litter was evaluated for viability at least twice daily. On PND 4 all litters were reduced to ten pups each by random selection; 25 appropriately sized litters per group were selected for examination on study. Ten pups of each sex and dosage group were randomly selected for brain weight/dimensions determination and neurohistopathology (Subsets 1 and 4). Litters with fewer than nine pups were not assigned to study, but retained until not needed. One male and one female pup/litter (up to 25/sex/subset) were assigned to each evaluation as follows:

   The pups in each litter were counted once daily. Clinical observations were recorded once daily during the preweaning period. Additionally, on PND 4 and 11, all pups in Subset 4 were examined outside of the home cage for *detailed* clinical observations. Observations were made at approximately the same time each day. The detailed clinical observations were documented by an observer unaware of the dosage group assignment of the pups. These observations included (but were not limited to): general appearance (skin, fur, changes in eyes, eyeballs and mucous membranes), body position and posture (e.g., hunchback posture), autonomic nervous system function (lacrimation, piloerection, pupil diameter, respiration, excretion), motor coordination, ambulatory abnormalities, reaction to being handled and to environmental stimulation, nervous system (tremor, convulsion, muscular contractions), changes in exploratory behavior, ordinary behavior (changes in grooming, head shaking, gyration), abnormal behavior (autophagia, backward motion, abnormal vocalization) and aggression. Pup body weights were recorded on PNDs 0, 4, 7, 11 , 13, 17 and 21.

2. **Developmental landmarks:** Male rats were evaluated daily for the age of preputial separation beginning on PND 38 and females beginning on PND 27. The age and body weight at onset was reported.

3. **Postweaning observations**: Rats were observed for viability at least twice daily during the postweaning period. These rats were also examined for clinical observations and general appearance once weekly during the postweaning period. Additionally, rats assigned to Subsets 2 and 3 were examined for gross signs of toxicity and clinical observations; and rats assigned to Subset 4 were examined by an individual unaware of each rat's dosage group for detailed clinical observations as previously described. Body weights for male and female rats were recorded weekly during the postweaning period and prior to sacrifice. Feed consumption values for male and female rats were recorded weekly during the postweaning period.
4. **Neurobehavioral evaluations:** Observations and the schedule for those observations are summarized as follows:
    i. **Motor activity:** Motor activity was evaluated on PND 13, 17, 21 and 60 through 62. One male and one female rat (when possible) from each litter were examined throughout the four testing periods. The movements of each rat were monitored by a passive infrared sensor mounted outside a stainless steel wire-bottomed cage (40.6 x 25.4 x 17.8 cm). (Plexiglas flooring was used on PNDs 13, 17 and 21). Each test session was 1.0 hour in duration with the number of movements and time spent in movement tabulated at each ten-minute interval. The apparatus was designed to monitor a rack of up to 32 cages and sensors during each session, with each rat tested in the same location on the rack across test sessions. Groups were counterbalanced across testing sessions and cages.
    ii. **Acoustic startle habituation:** Acoustic startle habituation was evaluated on PND 22 and 59 through 62. One male and one female rat (when possible) from each litter were examined for their reactivity to acoustic stimuli and habituation of responses with repeated presentation of stimuli. The rats were tested in sets of up to four within a sound-attenuated chamber. Each rat was placed inside a small cage situated above a platform containing a force transducer in its base. A microcomputer was used to sample the output of the force transducer and control the test session. The rats were initially given an adaptation period of five minutes. During the last minute of this period, ten "blank" trials were given to sample the baseline force in the absence of a stimulus. The rats were then presented with 30 msec, 120 dB bursts of noise at ten-second intervals for 50 trials. An additional ten "blank" trials followed. The peak amplitude of each response was recorded and the average response on baseline trials subtracted to calculate the response magnitude. The average response magnitude and the pattern of responses over ten trial blocks were compared among the dosage groups.
    iii. **Watermaze testing;** Beginning on PND 58 to 62, one male rat and one female rat (when possible) from each litter were evaluated in a water-filled M-maze for overt coordination, swimming ability, learning and memory. Each rat was tested in a watertight 16-gauge stain less steel modified M-maze. The maze was filled with water to a depth of approximately nine inches, and the water was monitored for temperature (range of 21°C± 1°C).

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On each test trial, the rat was placed into the starting position (base of the M-maze stem farthest from the two arms) and required to swim to one of the two goals of the M-maze, in order to be removed from the water. On the first trial, the rat was required to enter both arms of the maze before being removed from the water. The initial arm chosen on trial I was designated the incorrect goal during the remaining trials. Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal then removed from the water. A 15-second intertrial interval separated each trial. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in any test session was 15. Latency (measured in seconds) to choose the correct goal or the maximum 60-second interval was recorded for each trial, as was the number of errors (incorrect turns in the maze) during each trial.

Each rat was tested twice. The test sessions were separated by a one-week interval, and the correct goal and the criterion were the same for both test sessions. Dosage groups were compared for the following dependent measures; the number of trials to criterion on the first day of testing--this measure was used to compare groups for overall learning performance; the average number of errors (incorrect turns in the maze) for each trial on the first day of testing--this measure was used to compare groups for overall learning performance; the latency (in seconds) to reach the correct goal on trial 2 of the first day of testing--this measure was used to compare groups for short-term retention; the number of trials to criterion on the second day of testing--this measure was used to compare groups for long-term retention; the average number of errors for each trial on the second day of testing--this measure was used to compare groups for long-term retention; and the latency (in seconds) to reach the correct goal on trial 1 of day 2 of testing--this was another indicator of long-term retention.

iv. **Passive avoidance:** Beginning on PND 22 to 24, learning, short-term retention, long-term retention and hyperactivity were examined in a passive avoidance test. One male rat and one female rat (when possible) from each litter were tested.

Each rat was tested twice. The test sessions were separated by a one-week interval, and the criterion was the same for both days of testing. Dosage groups were compared for the following dependent measures: the number of trials to the criterion in the first session--this measure was used to compare groups for overall learning performance; the latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial I in the first test session--this measure was used to compare groups for activity levels and exploratory tendencies in a novel environment; the latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 2 in the first test session--this measure was used to compare groups for short-term retention; the number of trials to the criterion in the second test session--this measure was used

to compare groups for long-term retention; and the latency (in seconds) to enter the "dark" compartment from the "bright" compartment on trial 1 in the second session—this value was another indication of long-term retention.

2. **Postmortem observations:**
   a. **Maternal animals:** Rats were sacrificed by carbon dioxide asphyxiation, with the exception of rats selected for neurohistopathological evaluations; these rats were sacrificed by intravenous injection of sodium pentobarbital. Dams that delivered a litter and were selected for continued observation were sacrificed on PND 21. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed; the number and distribution of implantation sites were recorded. Rats that did not deliver a litter were sacrificed on DO 25 and examined for gross lesions. Uteri were examined while being pressed between glass plates to confirm the absence of implantation sites.
   b. **Offspring:** Pups were sacrificed by injection (*i.p.)* of sodium pentobarbital (≤ 14 days of age) or CO2 asphyxiation (>14 days of age). Subset 1 pups selected for brain weight/dimension determination and neurohistopathological evaluations were sacrificed and processed on PND 21. All pups in Subset 1 were examined for gross lesions. Subsets 2 and 3 rats were sacrificed after completion of the postweaning behavioral evaluations and examined for gross lesions. Subset 4 rats randomly selected for brain weight/dimensions determination and neurohistopathological evaluations were sacrificed on PND 69 and those not selected for these procedures were sacrificed and examined for gross lesions. Subset 5 pups were sacrificed on PND 21 and examined for gross lesions.

   Pups that died before scheduled termination were examined for gross lesions and the cause of death as soon as possible after the observation was made. Pups found dead on PND 1 to 4 were preserved in Bouin's solution for possible future evaluation. All offspring not selected for continued evaluation were necropsied prior to weaning (PND 21) and gross lesions were retained in Bouin's solution. All gross lesions were submitted for histological evaluation.

   Neurohistopathology and morphometrics: Ten FI rats/sex/dose group at each of two time points - postnatal day (PND) 22 and approximately PND 70 (adults) were perfusion-fixed and tissues saved or prepared for neuropathologic evaluation. Thorough histopathological examinations were performed on the brains of the PND 22 rats and on both the central and peripheral nervous systems of the adult rats in each of the 0 and 10,000 ppm dietary groups.

   Brain sections from the PND 22 rats were stained with hematoxylin and eosin (H&E) and with the luxol fast blue/cresyl violet (LFB/CV) stains. All paraffin-embedded tissues from the 70 adult rats were stained with H&E, LFB/CV, and with the Bielschowsky's technique (a silver stain for axons and neuronal cytoarchitecture). Sections from the glycol methacrylate blocks were stained with hematoxylin and eosin, toluidine blue, and the Bielschowsky's technique.

   The block list for the evaluated tissues is as follows:
   Paraffin-embedded tissues:
   Block 1: Coronal slice through the cerebrum at the level of the optic chiasm.
   Block 2: Coronal slice through the cerebrum at the level of the infundibulum.
   Block 3: Coronal slice through the cerebrum at the level of the mammillary bodies.

Block 4: Coronal slice through the middle of the cerebellum.
Block 5: Multiply-embedded sections including the olfactory bulbs, two coronal slices through the anterior pole, one coronal slice through the cerebrum at the level of the midbrain, one through the posterior portion of the cerebellum, and one through the medulla oblongata.
Block 6: Longitudinal sections of the Gasserian ganglia and associated trigeminal nerves.
Block 7: Eyes.
Block 8: Transverse and oblique sections of the cervical, thoracic, and lumbar spinal cord.
Block 9: Skeletal muscle.

Glycol methacrylate-embedded tissues:
Block 10: Cervical, thoracic and lumbar dorsal root ganglia with dorsal spinal nerve roots (longitudinal).
Block 11: Sciatic nerve, proximal (longitudinal and transverse).
Block 12: Tibial nerve, proximal (longitudinal and transverse).
Block 13: Fibular (peroneal) nerve (longitudinal and transverse).
Block 14: Sural nerve (longitudinal and transverse).
Block 15: Cervical, thoracic and lumbar ventral spinal nerve roots (longitudinal).

Detailed morphometric evaluation included the following measurements:
Thickness of the frontal cortex;
Diagonal width of the caudate putamen and underlying globus pallidus;
Thickness of corpus collosum;
Thickness of hipocampal gyrus;
And maximum height of cerebellum

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No adverse clinical observations related to exposure to dinotefuran occurred. All clinical and detailed clinical observations that occurred during the gestation and lactation periods were considered unrelated to the test substance because:
1) the findings occurred in the control group only;
2) the observation occurred in only one to three rats in an exposure group;
3) the incidence was not dosage-dependent; and/or
4) the observation(s) did not persist.

These clinical observations included sparse hair coat; scab on the mouth, tail, tip of tail or lower midline; tip of tail missing; tip of tail red or black; bent tail; hyperactivity; a torn ear; localized alopecia (limbs, head, neck and/or underside); ungroomed coat; urine-stained abdominal fur; soft or liquid feces; a mass in the axilla; chromorhinorrhea; red perivaginal substance; hunched posture; lacrimation; chromodacryorrhea; limited use of the right forelimb; and piloerection.

Statistically significant increases in umbilical hernia (3000 ppm, subset 4) and injured tails (1000 ppm) were observed but not regarded as test article-related.

2. **Mortality:** All F0 generation female rats survived to scheduled sacrifice. No mortality related to maternal exposure to MTI-446 occurred in the male or female rats postweaning. All male rats survived to scheduled sacrifice. One F1 female rat in the 10000 ppm maternal exposure group was found dead on PND 23, another was found dead on PND 26 and two female rats in the 0 ppm exposure group was missing on PND 22 and another was sacrificed due to adverse clinical observations on PND 58. These single events are considered incidental. All other rats survived to scheduled sacrifice.

3. **Necropsy Observations:** A summary of the necropsy performed on generation F0 is provided below.

Table 2 – Necropsy Observations – F0 Generation Female Rats

DOSAGE GROUP	I	II	III	IV
CONCENTRATION (PPM)[a]	0	1000	3000	10000
RATS EXAMINED[b]	25	25	25	25
MORTALITY	0	0	0	0
APPEARED NORMAL	24	25	23	23
MAMMARY TISSUE - Firm	0	0	1	0
STOMACH : MUCOSAL SURFACE, RED AND THICK	1	0	1	1
KIDNEYS : RIGHT, PELVIS, SLIGHT DILATION	0	0	0	1

a. Rats were given continuous access to the test substance in the diet from day 6 of presumed gestation until sacrifice (day 21 of lactation).
b. Refer to the individual clinical observations table (Table A16) for external observations confirmed at necropsy.
Source: Table A3, p. 86 of the Study Report.

B. **BODY WEIGHT AND BODY WEIGHT GAIN:** Body weight gains for days 6 to 9 of gestation (GD 6 to 9) and GD 6 to 20 were significantly reduced ($p \leq 0.01$) in the 10000 ppm exposure group compared to the control group value. Mean body weight gain during the gestation period (GD 6 - 20) was 11.2% lower than the control group value. However, mean body weights were generally comparable among the dosage groups and body weights on GD 20 were 98.7%, 99.7% and 96.8% in the 1000, 3000 and 10000 ppm exposure groups, respectively, compared to the 0 ppm exposure group.

Table 3 - Maternal body Weights – Gestation – Summary – F0 Generation Female Rats

Dosage Group		I	II	III	IV
Concentration (PPM) [a]		0	1000	3000	10000
Rats Tested	N	25	25	25	25
Pregnant	N	23	25	23	24
Maternal ~ Body Weight Mean (g) ± SD					
Day 0	Mean ± S.D.	235.3 ± 9.1	234.5 ± 10.5	235.4 ± 9.4	235.5 ± 9.6
Day 6	Mean ± S.D.	270.3 ± 11.8	269.0 ± 11.0	273.0 ± 11.0	271.5 ± 13.2
Day 7	Mean ± S.D.	273.8 ± 12.2	272.7±13.4	274.2 ± 12.4	268.5 ± 13.9
Day 8	Mean ± S.D.	278.7 ± 13.2	277.9 ± 13.3	280.5 ± 12.9	273.2 ± 13.9
Day 9	Mean ± S.D.	284.4 ± 13.0	283.7 ± 14.0	285.1 ± 12.3	279.2 ± 13.2
Day 10	Mean ± S.D.	290.5 ± 13.	289.2 ± 14.9	291.3 ± 12.8	284.2 ± 13.4
Day 11	Mean ± S.D.	297.3 ± 12.5	296.5 ± 15.4	297.3 ± 14.4	291.0 ± 13.0
Day 12	Mean ± S.D.	302.0 ± 14.1	301.1 ± 17.2	303.4 ± 14.2	296.4 ± 14.0
Day 13	Mean ± S.D.	307.9 ± 15.4	306.2 ± 16.3	309.4 ± 15.5	301.0 ± 14.0

Day 14	Mean ± S.D.	313.4 ± 14.6	310.3 ± 18.0	316.3 ± 15.5	307.0 ± 14.4
Day 15	Mean ± S.D.	322.8 ± 16.9	318.8 ± 18.5	324.8 ± 16.6	315.9 ± 16.0
Day 16	Mean ± S.D.	333.3 ± 16.7	328.8 ± 19.5	334.3 ± 17.5	324.3 ± 16.5
Day 17	Mean ± S.D.	345.7 ± 16.8	339.3 ± 20.8	346.6 ± 19.6	336.9 ± 18.1
Day 18	Mean ± S.D.	360.4 ± 18.2	355.4 ± 21.3	361.2 ± 19.9	350.0 ± 19.2
Day 19	Mean ± S.D.	375.3 ± 20.6	369.1 ± 21.2	374.8 ± 21.4	363.0 ± 20.5
Day 20	Mean ± S.D.	390.6 ± 22.4	385.4 ± 23.8	389.5 ± 22.9	378.3 ± 22.7

[a] – Rats were given continuous access to the test substance in the diet from day 6 of gestation until sacrifice (day 21 of lactation).
Source: Table A4, p. 87 of the Study Report.

Table 4 - Maternal body Weight Changes – F0 Generation Female Rats

Dosage Group		**I**	**II**	**III**	**IV**
Concentration (PPM) [a]		**0**	**1000**	**3000**	**10000**
Rats Tested		N	25	25	25
Pregnant		N	23	25	23
Maternal ~ Body Weight Change Mean (g) ± SD					
Days 0 - 6	Mean ± S.D.	+34.9 ± 6.7	+34.5 ± 7.4	+37.6 ± 7.6	+36.0 ± 7.9
Days 6 - 9	Mean ± S.D.	+14.2 ± 3.9	+14.8 ± 4.7	+12.0 ± 3.8	+7.7 ± 4.9**(↓45%)
Days 9 - 12	Mean ± S.D.	+17.5 ± 4.5	+17.4 ± 5.2	+18.3 ± 3.2	+17.2 ± 4.8
Days 12 - 15	Mean ± S.D.	+20.9 ± 5.7	+17.7 ± 3.9	+21.4 ± 5.8	+19.5 ± 6.1
Days 15 - 18	Mean ± S.D.	+37.6 ± 5.0	+36.6 ± 6.2	+36.4 ± 6.6	+34.1 ± 8.0
Days 18 - 20	Mean ± S.D.	+30.1 ± 8.7	+30.0 ± 6.4	+ 28.3± 5.8	+28.3 ± 5.5
Days 6 - 20	Mean ± S.D.	+120.3 ± 15.9	+116.4 ± 15.9	+116.4 ± 15.0	+106.8 ± 13.9** (↓11%)
Days 0 - 20	Mean ± S.D.	+155.2 ± 19.9	+150.9 ± 19.9	+154.1 ± 19.4	+142.8 ± 20.0

[a] – Rats were given continuous access to the test substance in the diet from day 6 of gestation until sacrifice (day 21 of lactation).
** Significantly different from the Group I value ($P \leq 0.01$).
Source: Table A5, p. 88 of the Study Report.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** Absolute (g/day) and relative (g/kg/day) food consumption (measured only during the post dosage period) was not affected by maternal exposure to MTI -446 at exposure levels as high as 10000 ppm.

2. **Test substance consumption:** Absolute food consumption values in the 3000 and 10000 exposure Subset 4 group were significantly increased $p \leq 0.05$) PNDs 63 to 69 compared to the control group value. Relative food consumption values in the 1000 and 10000 ppm exposure Subset 2 groups were significantly increased ($p \leq 0.05$ and $p \leq 0.01$, respectively) on PNDs 63 to 71 compared to the control group value.

   Absolute feed consumption values in the 1000 and 10000 ppm exposure group Subset 4 female rats were significantly increased ($p \leq 0.05$) on PNDs 49 to 56, compared to the control group value. Relative feed consumption values in the 1000 ppm exposure group Subset 2 female rats was significantly increased ($p \leq 0.05$) on PNDs 49 to 56, compared to the control group value.

   These findings are not considered adverse in the absence of food efficiency data or significant body weight loss

3. **Food efficiency:** Food efficiency was not determined.

D. HISTOPATHOLOGY AND NEUROPATHOLOGY

1. **Gross pathology:** All necropsy observations for the F1 generation male and female rats were considered to be unrelated to the test substance because:
   1) the incidences were not dosage-dependent;
   2) the observations occurred in only one or two rats per subset; and/or
   3) these observations commonly occur in this strain of rat.

   Macroscopic observations included: constricted area of the liver, slight to moderate dilation of the renal pelvis, numerous tan areas on the kidneys, a mass on the epididymis, small and flaccid testes, large testes, small epididymis, three white lobular masses on the testis, two masses in the adipose tissues proximal to the testis, situs inversus, numerous red areas on the thymus, accessory spleen and a misshapen kidney.

2. **Brain Measurements:** Brain weight, cerebrum and cerebellum length were comparable for the male and female rats in all groups on days 21 and 69 postpartum. No significant differences occurred among the groups, with the exception of the 1000 ppm males on PND 21 that showed a significant $p \leq 0.05$ lower mean brain weight then controls. Mean brain weights in males at the 3000 or 10000 ppm were comparable to the control group value (Table 5).

Table 5 – F1 Generation Brain Measurements

MATERNAL DOSAGE GROUP	I	II	III	IV
MATERNAL CONCENTRATION (PPM)	0	1000	3000	10000
Subset 1 (Sacrificed on Day 21 Postpartum)				
MALE RATS				
RATS TESTED	10	10	10	10
BRAIN WEIGHT (G)	1.71477 ±0.13039	1.59363±0.13359*	1.75202±0.09615	1.72599±0.09923
CEREBRUM LENGTH (MM)	14.01±0 .38	13.74± 0.39	13.93± 0.34	13.92 ±0.27
CEREBELLUM LENGTH (MM)	6.69±0 28	6 .68 ± 0.36	6 .57 ± 0 .35	6.84 ± 0.26
FEMALE RATS:				
RATS TESTED	10	10	10	10
BRAIN WEIGHT (G)	1.6867±0.11323	1.60382±0.13729	1.67212±0.12136	1.69252±0.0628
CEREBRUM LENGTH (MM)	13.77 ± 0.31	13.59± 0.38	13.80 ± 0.38	13.52± 0.27
CEREBELLUM LENGTH (MM)	6.45±0.49	6.58±0.30	6.61±0.38	6.66±0.31
Subset 4 (Sacrificed on Day 69 Postpartum)				
MALE RATS				
RATS TESTED	10	10	10	10
BRAIN WEIGHT (G)	2.21763±0.14294	2.26989±0.14244	2.29016±0.12354	2.29492±0.09384
CEREBRUM LENGTH (MM)	15.65±0.20	15.82±0.33	15.64±0.34	15.76±0.31
CEREBELLUM LENGTH (MM)	6.88±0.32	7.11±0.31	7.08±0.48	7.07±0.29
FEMALE RATS:				
RATS TESTED	10	10	10	10
BRAIN WEIGHT (G)	2.03122±0.12406	2.09004±0.10142	2.07120±0.1105	2.03187±0.12535
CEREBRUM LENGTH (MM)	15.32±0.40	15.27±0.47	15.28±0.32	15.18±0.24
CEREBELLUM LENGTH (MM)	6.61±0.37	6.74±0.39	6.71±0.28	6.44±0.36

* Significantly different from Group I value ($p \leq 0.05$).
Source: Tables B5, p. 228-229 of the Study Report.

3. **Histopathology:** There were no microscopic changes in the tissues evaluated histologically that were considered to have been the result of test substance administration. The changes that were observed were typical of those that occur spontaneously or as incidental findings in rats of this age and strain.

4. **Motor Activity:** Motor activity evaluated on PNDs 13, 17, 21 and 60 ± 2 was not affected by maternal exposures to MTI-446 as high as 10000 ppm. No statistically significant differences occurred among the groups for either the number of movements or time spent in movement (Table 6).

Table 6 – Summary of Motor Activity in F1 Female Rats

Day 60 Postpartum (±2 Days)				
Maternal Dose Group	**I**	**II**	**III**	**IV**
Maternal Concentration	**0**	**1000**	**3000**	**10000**
Number of movements				
Number of Rats	20a	21	20	20b
BLOCK 1	126.4±15.3	127.7±17.9	125.6±16.1	133.3±11.2
BLOCK 2	134.3±15	141.5±15.9	133.6±11.6	141.2±12.8
BLOCK3	143±12.5	147.1±25.2	139.9±12.9	149.8±26.5
BLOCK 4	124.9±40.3	139.7±40.6	141.1±22.7	136.4±37.5
BLOCK 5	111.8±52.2	127.7±52.6	122.1±50.5	127.7±44.3
BLOCK 6	102.7±58	113.4±57.2	114.3±48.9	99.6±66.1
TOTAL	743±128.4	797.1±134.5	776.4±105.8	787.9±116.1
Time (s) spent in movement				
BLOCK 1	424.6±61.5	422.3±69.6	421.3±66.4	413.9±40.6
BLOCK 2	342.5±78.9	343.5±63.6	337±76.2	337.7±52.9
BLOCK 3	312.9±72.5	298.7±69.0	299.2±77.3	295±65.5
BLOCK 4	247.4±121.0	252±98.3	253±99.4	243.8±92.4
BLOCK 5	196.8±122.6	219.7±108.6	210.5±110.7	214.7±105.8
BLOCK 6	166.7±113.5	195.9±126.5	202.0±116.6	147.3±103.6
TOTAL	1690.8±468.5	1732.2±350.4	1722.9±439.8	1652.3±282.3

a – Excludes rat 3408
b – Excludes rat 9408
Source: Table B16, p. 262 of the Study Report.

5. **Acoustic Startle Habituation:** Acoustic startle habituation evaluated on PNDs 22 and 60 ± 2 was not affected by maternal exposure to MTI-446 as high as 10000 ppm. No statistically significant differences occurred among the groups for either the pattern of response magnitudes or average response magnitude.

Table 7 – Acoustic Startle Summary

F1 Generation Male Rats				
DAY 22 POSTPARTUM (±2DAYS)				
Maternal Dose Group	I	II	III	IV
ppm	0	1000	3000	10000
	g	g	g	g
Number of Rats	21	21	20	21
BLOCK 1	13.44±7.2	20.38±13.83	13.05±6.17	15.94±10.69
BLOCK 2	9.39±6.54	11.45±7.71	10.24±7.99	10.38±6.86
BLOCK3	8.14±3.92	11.66±8.29	7.45±4.8	9.82±6.04
BLOCK 4	7.47±5.01	9.91±8.30	10.02±8.57	10.2±6.77
BLOCK 5	9.03±6.37	11.41±8.17	11.31±11.52	13.15±12.47
AVERAGE	9.49±4.53	12.97±8.54	10.41±6.67	11.90±7.56
DAY 60 POSTPARTUM (±2DAYS)				
Number of Rats	21	20	20a	20a
BLOCK 1	46.23±35	60.73±44.71	53.15±33.55	55.28±29.68
BLOCK 2	25.84±27.02	34.30±29.92	38.64±34.76	27.79±29.67
BLOCK3	20.44±21.26	26.70±18.69	29.73±24.59	21.46±18.21
BLOCK 4	18.87±21.94	29.03±25.62	23.36±27.69	18.26±17.38
BLOCK 5	16.60±22.19	26.04±25.54	22.75±17.26	20.79±22.06
AVERAGE	25.59±23.12	35.35±25.44	33.52±24.9	28.71±18.97
F1 Generation Female Rats				
DAY 22 POSTPARTUM (±2DAYS)				
Number of Rats	20a	21	20	21
BLOCK 1	11.28±7.48	15.10±7.56	14.60±7.77	14.38±11.16
BLOCK 2	7.56±5.76	9.56±8.00	11.66±6.11	9.77±9.73
BLOCK3	8.23±6.04	10.76±8.17	10.36±7.62	9.22±8.39
BLOCK 4	10.49±8.78	11.85±12.26	10.06±7.34	11.47±10.27
BLOCK 5	12.81±9.84	10.94±8.49	12.63±9.82	14.73±12.57
AVERAGE	10.07±5.87	11.64±7.41	11.86±6.39	11.91±8.45
DAY 60 POSTPARTUM (±2DAYS)				
Number of Rats	20b	21	20	20c
BLOCK 1	21.11±14.59	25.78±16.94	35.52±24.88	27.34±12.84
BLOCK 2	10.94±6.34	11.44±11.48	22.40±21.92	16.87±9.34
BLOCK3	9.43±8.67	16.08±13.21	18.59±17.43	15.17±16.58
BLOCK 4	12.43±13.9	15.31±15.53	13.92±16.72	10.60±16.1
BLOCK 5	10.82±10.02	10.57±8.03	17.57±21.55	10.73±7.3
AVERAGE	12.95±8.66	15.83±10.35	21.59±19.00	16.14±9.54

a – Excludes value of rat 7303
b - Excludes value of rat 3408
c - Excludes value of rat 9408
Source: Table B17, p. 263-264 of the Study Report.

6. **Passive Avoidance and Watermaze Performance:** In the passive avoidance test, the mean number of trials to response, the latency of response and the numbers of rats failing to learn were comparable in all treated and control groups and none of the values for the treated groups was significantly different from the control values; with the exception of the latency in the female 1000 ppm group during the second trial of the learning phase which was significantly higher *($p \leq 0.05$)* than the other treated groups and the control group. This effect was not considered related to the test substance because it was not dosage dependent (Table 8).

Table 8 – F1 Generation Passive Avoidance Performance

Maternal Dosage Group	I	II	III	IV
Maternal Concentration (ppm)	**0**	**1000**	**3000**	**10000**
Male Rats				
Session 1a	21	21	20	21
Trials to Criterion	6.3±3.5	5.5±3.1	5.4±1.7	6.2±3.0
Latency Trial 1b (sec)	4.5±2.7	5.9±4.1	4.9±2.6	5.8±7.0
Latency Trial 2b (sec)	18.7±17.2	25.3±21.9	22.4±21.0	23.0±20.4
Failed to Learn c	9.5%	4.8%	0.0%	4.8%
Session 2a	19	20	20	20
Trials to Criterion	3.5±2.8	2.8±0.6	3.0±0.5	3.0±0.6
Latency Trial 1b (sec)	28.8±22.8	30.7±23.5	26.4±21.7	25.6±22.1
Female Rats				
Session 1a	20d	21	20	20d
Trials to Criterion	5.2±1.6	4.7±1.1	5.0±1.5	5.2±1.9
Latency Trial 1b	4.3±3.0	4.7±3.7	4.8±3.4	4.0±5.5
Latency Trial 2b	21.6±18.0	35.4±22.0*	22.1±20.2	19.0±19.6
Failed to Learn c	0.0%	0.0%	0.0%	0.0%
Session 2a	20d	21	20	20d
Trials to Criterion	3.0±0.6	3.4±1.9	3.8±2.8	3.2±0.8
Latency Trial 1b	25.8±23.9	31.3±22.8	27.1±24.9	31.7±24.6

a - Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one- week interval.
b - The latency was recorded in seconds.
c - Number of rats that did not meet the criterion in Session 1 (Learning Phase); Session 2 (Retention Phase) values for these rats were excluded from summarization and statistical analyses.
d - Excludes values for rats that were missing or found dead.
* Significantly different from the Group I value ($p \leq 0.05$).
Source: Table B19, p. 265 of the Study Report.

In the watermaze performance assessment, the number of trials to achieve criterion, errors/trial, the numbers of rats failing to learn and the latency periods were comparable in all treated and control groups and none of the values for the treated groups was significantly different from the control values (Table 9).

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Table 9 – F1 Generation Watermaze Performance

Maternal Dosage Group	I	II	III	IV
Maternal Concentration (ppm)	**0**	**1000**	**3000**	**10000**
Male Rats				
Session 1a	21	21	20	21
Trials to Criterion	9.6±3.2	9.4±2.7	8.8±3.0	7.8±1.7
Errors per Trial	0.46±0.30	0.46±0.23	0.36±0.18	0.34±0.15
Latency Trial 2b (sec)	15.8±13.8	16.6±12.6	13.4±6.0	17.9±12.9
Failed to Learn c	9.5%	4.8%	10.0%	0.0%
Session 2a	19	20	18	21
Trials to Criterion	6.0±1.9	6.9±2.8	8.0±3.4	7.0±3.0
Errors per Trial	0.06±0.10	0.15±0.19	0.5±0.17	0.16±0.23
Latency Trial 1b (sec)	8.3±4.2	10.8±7.4	10.0±5.9	8.9±6.1
Female Rats				
Session 1a	20d	21	20	20d
Trials to Criterion	9.0±2.3	9.8±2.7	9.8±2.9	9.4±3.3
Errors per Trial	0.39±0.18	0.41±0.20	0.48±0.24	0.44±0.22
Latency Trial 2b	17.2±13.0	15.8±12.5	14.6±8.0	16.5±13.5
Failed to Learn c	0.0%	0.0%	5.0%	10.0%
Session 2a	20d	21	19	18d
Trials to Criterion	6.8±2.1	7.7±3.0	6.9±2.6	7.3±3.1
Errors per Trial	0.13±0.14	0.15±0.17	0.15±0.19	0.11±0.15
Latency Trial 1b	11.6±6.2	10.8±4.0	12.5±7.7	10.4±6.1

a . Sessions 1 (Learning Phase) and 2 (Retention Phase) of testing were separated by a one-week interval.
b . The latency was recorded in seconds.
c . Number of rats that did not meet the criterion in Session 1 (Learning Phase) ; Session 2 (Retention Phase) values for these rats were excluded from summarization and statistical analyses.
d . Excludes values for rats that were missing or found dead.
Source: Table B20, p. 266 of the Study Report.

7. **Morphometry and Neuropathology:** No statistically significant inter-group differences were noted at any dose level for brain weights or for gross cerebral and cerebellar measures in 21 day old and adult rats. In addition, none of the microscopic brain measurements (frontal cortex, parietal cortex, striatum, corpus callosum, hippocampus and cerebellum) differed significantly between the control and high dose (10000 ppm) groups for either 21 day old or adult rats. Finally, no treatment-related microscopic lesions were present in any of the tissues examined in the central and peripheral nervous systems at 10000 ppm. Therefore, the high dose group of 10000 ppm ofMTI-446 in the maternal diet, under the conditions of this study, is considered to represent a no-observed adverse-effect level (NOAEL) for developmental histopathological neurotoxicity in the rat (Table 10).

Table 10 – Mean Microscopic Brain Measurements of Rats

Animal ID	Frontal Cortex (µm)	Parietal Cortex (µm)	Striatum (Caudate-Putamen) (µm)	Corpus Callosum (µm)	Hippocampus (µm)	Cerebellum (µm)
Measurements in PND 22 Male Rats at 0 ppm						
Mean	2088	2088	2970	247	1404	5148
SD	142.27	113.31	139.28	31.09	74.56	267.47
Measurements in PND 22 Male Rats at 10,000 ppm*						
Mean	2097	2040	2946	247	1362	5127
SD	149.97	107.7	77.2	26.05	63.56	368.78
Measurements in PND 22 Female Rats at 0 ppm						
MEAN	2013	1998	2832	235	1302	5010
SD	119.54	83.9	83.9	42.41	56.92	295.63
Measurements in PND 22 Female Rats at 10,000 ppm						
MEAN	2073	2016	2922	227	1326	5220
SD	97.42	62.93	106.02	24.95	83.43	268.33
Measurements in Adult Male Rats at 0 ppm						
MEAN	1947	1971	2934	256.8	1389	5376
SD	84.202	93.86	121.49	37.18	81.3	262.6
Measurements in Adult Male Rats at 10,000 ppm**						
MEAN	1968	2016	3000	268	1425	5592
SD	80.25	39.5	74.83	32.86	75.17	249.48
Measurements in Adult Female Rats at 0 ppm						
MEAN	1908	1923	2940	256.8	1425	5112
SD	88.54	79.31	63.25	31.6	57.01	225.92
Measurements in Adult Female Rats at 10,000 ppm						
MEAN	1947	1959	2952	254.4	1380	5292
SD	75.43	78.8	61.97	27.6	64.81	234.61

*The cerebellar measure for Rat #7301 was not included due to an inaccurate cut.
**The corpus callosum measurement for Rat #2804 was not included due to excess hydrocephalus
No significant differences were present between the high dose group and the control group.
Source: Tables 9-12of pages 708-711 of the study report

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The no-observed-adverse-effect level (NOAEL) for both functional and histopathological developmental neurotoxicity in the Fl generation rats whose mothers were exposed to dinotefuran was 10000 ppm, the highest dose tested. This dietary concentration provided an average maternal dose level of 784 mg/kg/day during the gestation (GD 6 to 20) and 1643 mg/kg/day during the lactation period (PND 0 to 21).

A NOAEL for all toxicological effects was established as 3000 ppm (equivalent to an average maternal dose level of 237 mg/kg/day during the gestation and 501 mg/kg/day during the lactation period) based on the occurrence of reduced maternal body weight gain during gestation at 10000 ppm.

B. REVIEWER COMMENTS: The reviewer agrees that the NOAEL for both functional and histopathological developmental neurotoxicity in the F1 generation rats whose mothers were exposed to MTI-446 was 10000 ppm, the highest dose tested. This dietary concentration provided an average maternal dose level of 784 mg/kg/day during the gestation and 1643 mg/kg/day during the lactation period. However the decreases in body weight gain are not considered adverse since they are not sustained and there is no difference in body weight among dose groups during gestation , lactation or postweaning. Therefore, a NOAEL for all toxicological effects is also established as 10000 ppm, the highest dose tested.

C. STUDY DEFICIENCIES: There were no major study deficiencies in study design or methods.

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